Ligation

Rationale:	
Special Observations:	
Results:	
Interpretation :	

Experiment Date:
Experiment Time:
Primary Experimenter (contact):
Other Experimenters:

Source: NEB, Dujduan Waraho

Assembled: 6/27/2012

Reagent	Details	Quantity
ddH2O (nuclease-free)		Bring total vol. to 20 µL
10X T4 DNA Ligase Buffer*		2 µL
Vector DNA		**Vector : Insert = 1 : 3
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T4 Ligase		1 µL

*Thawed and resuspended at room temperature
** 1:3 molar ratio of vector : insert (but it can vary from 1:2 to 1:6);

Calculations:
Start with 10 ul vector and calculate volume of insert, then scale sum to ≤17uL.

$$\boldsymbol{Y}\ ng\ insert\ =\ (3) * \left(\boldsymbol{X}\ ng\ vector\right) * \frac{bp\ length\ of\ insert}{bp\ length\ vector}$$

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$$\boldsymbol{uL}\ insert\ to\ use = \frac{\boldsymbol{Y}\ ng\ insert}{insert\ conc.\ in \frac{ng}{uL}}$$

$$\boldsymbol{uL}\ vector\ to\ use = \frac{(\boldsymbol{X}\ ng\ vector)}{vector\ conc.\ in \frac{ng}{uL}}$$

Procedure:

Critical Steps:

Label microcentrifuge tubes, put on ice

Add components in listed order to microcentrifuge tubes on ice

For numerous ligations, prepare mastermix with water and buffer

Incubate at 4°C overnight and/or room temperature for at least 30 minutes

Chill on ice and transform 5 µl of the reaction into 50 µl competent cells.

- See electroporation protocol